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(54) Title: IMMORTALIZED CELLS AND USES THEREFOR

(57) Abstract

The present invention provides immortalized tick cell lines, an immortalized bovine T cell line and uses therefor, including diagnostic reagents and vaccine compositions.

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IMMORTALIZED CELLS AND USES THEREFOR

Field of the Invention

This invention relates generally to immortalized primary cells, and, more specifically, to immortalized cell lines useful for the production of antigens and biologically active compounds.

Background of the Invention

Continuous or immortalized cell lines which are characterized by the ability to grow indefinitely are valuable as a source of immunogens, a source of new biologically active compounds and for the production and replication of relevant pathogens. The ability to establish a culture that will grow indefinitely varies depending on the animal species from which the cells originate. For example, rodent cells routinely generate continuous cell lines, but chicken cells almost never become immortal. Human cells, with the exception of tumor cells, are not good sources of immortalized cell lines.

Tick cell cultures are of interest for the research and study of tick-borne pathogens and microorganisms. Presently available in vitro primary or continuous tick cell cultures are not capable of long term, consistent maintenance of tick-borne pathogens.

There exists a need in the art for immortalized in vitro cell cultures of cells capable of supporting the growth of pathogenic organisms, for use in developing vaccines to selected pathogens and for research purposes.

Summary of the Invention

The invention provides immortalized cultures of primary cells. These cell lines are useful, for example, as antigens in vaccine compositions, as vessels for growth and production of antigens, as diagnostic reagents, and in therapeutic compositions.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Detailed Description of the Invention

The present invention provides immortalized tick cell lines and an immortalized bovine T cell line. Such immortalized cell lines according to this invention are useful as, or in the production of, vaccinal agents, e.g., against ticks, and may provide protection against diseases caused by pathogens, e.g., tick-borne pathogens.

As used herein, the term "immortalized cell line" refers to the cells deposited with the American Type Culture Collection (ATCC) as stated below. Immortalized cells of this invention may be clonally expanded by conventional techniques to produce a homogeneous population of progeny cell lines which can be maintained continuously in an appropriate culture medium.

Immortalized cultures of Dermacentor andersoni and Amblyomma americanum tick gut are provided. One example is the tick cell line called DGEC-1. This cell line, is described in detail and characterized in Example 1. The line has remained stable for over 26 months and was deposited with the American Type Culture Collection (ATCC) on July 8, 1992 [ATCC Accession No. CRL 11084]. Also deposited with with ATCC on July 8, 1992 [ATCC Accession No. CRL 11083], was the AGEC-1 immortalized tick cell line of the invention, which is discussed below in more detail.

Further, the invention provides an immortalized bovine T cell line, designated Bpbl-T1 (bovine peripheral blood lymphocyte-T cell). This immortalized bovine T cell line is useful, inter alia, for T-cell dependent antigen targeting assays, T-cell receptor and antigen recognition studies, adoptive transfer experiments, and in vitro screening of cytokines, antigens and/or other bioresponse modifiers. This line has remained stable for over 8 months and was deposited with the ATCC on September 11, 1992 [ATCC Accession No. CRL11120].

This invention further includes progeny and derivatives of these cell lines, e.g., cells which have been derived from the specific identified cell line by passaging or clonal expansion. All statements made herein relating to the immortalized cell lines of the invention are equally applicable to their progeny and derivatives.

Immortalized tick cell cultures of this invention are useful for, among other things, growing, in vitro, tick-borne pathogens, such as Borrelia burgdorferi, the causative agent of Lyme disease, and for producing antigens to these pathogens. These cell lines have also proven useful in maintaining in vitro cultures of A. marginale, Ehrlichia species, and Borrelia burgdorferi.

The immortalized T cells of the invention are also useful for growing a selected pathogen, as well as for producing antigens to these pathogens. Additionally, the immortalized T cells of the invention are useful for the study of pathogens capable of infecting T cells, e.g. lentiviruses, and the study of T-cell signal transduction.

The immortalized cells of this invention provide a system for mass production of antigens, e.g., preferably cellular components such as proteins or non-proteinaceous material capable of inducing an immune response directed against a

parasite borne pathogen. According to one embodiment, an immortalized cell of the invention may itself produce a biological or therapeutic agent, e.g., a tick cell polypeptide or protein, or another component or fraction of a tick cell which enhances the ability of a vaccine composition containing a selected pathogenic antigen to stimulate a protective immune response in the vaccine. For example, the immortalized tick cell lines, AGEC-1 or DGEC-1, when cultured may naturally produce peptides, polypeptides, proteins, or other cellular fractions which are useful as anti-coagulants, anti-inflammatory agents and diuretics, for pharmaceutical and veterinary purposes. These exemplary and other agents are among the biological products naturally produced by an immortalized cell of this invention upon culture. Appropriate culture conditions to obtain maximum production of such natural products can be determined by one of skill in the art. These biological materials may be produced intracellularly and obtained from the cultured tick cell by conventional cell disruption, e.g., lysis, and purification of the material or a cellular fraction containing it from the lysate, based on its chemical identity or biological activity. Alternatively, the immortalized cell of the invention may secrete the agent into the media. Methods of isolating and purifying such biological materials or cell fractions are known in the art and may be utilized as desired.

According to another embodiment, the present invention provides a method for producing antigens directed against a selected pathogen by infecting an immortalized cell culture of the invention with a selected pathogenic microorganism, e.g., Borrelia burgdorferi, Ehrlichia canis, Anaplasma marginalis, Babesia bovis, Theileria parva. The infected immortalized cell may, upon culturing permit the natural replication of the pathogen, and the production of pathogenic proteins in culture. Either the pathogen itself, whether it be a virus or microorganism, or desirable proteinaceous materials including subunits, polypeptides, cell fractions, fragments thereof, or other macromolecules such as carbohydrates, lipids, and lipoproteins may be produced in, and isolated from, the immortalized cell culture by conventional biological and genetic engineering techniques. Such proteinaceous and non-proteinaceous materials, including the immortalized cell or pathogen itself, are defined herein as antigens. Alternatively, the pathogens may be co-harvested from the cell culture infected by the pathogen.

It is understood that proteinaceous and non-proteinaceous materials produced by the pathogen-infected immortalized cell lines may be include materials produced from the immortalized cell biosynthetic activity. Pathogen-produced materials isolated from the cell culture are expected to have use in vaccine compositions. For example, association of such pathogen-produced material with tick cell derived

material may provide vaccinal compositions with enhanced ability to stimulate immunity in the vaccinee due to the influence of the tick cell environment upon the development and growth of the pathogen in the tick cell culture. In a similar manner, the immortalized bovine cell line and products produced therein may be used to enhance vaccine compositions containing bovine pathogens or antigenic materials thereof. These cells and materials may further be of use in aiding identification of bovine antigens and in assaying for bovine cytokine production and activity.

Still an alternative embodiment for producing desirable antigens or polypeptides using an immortalized cell line of this invention involves transfecting the cell line with a recombinant molecule containing a heterologous polypeptide or protein having desirable antigenic properties under the control of a suitable regulatory sequence capable of directing the replication and expression thereof in the immortalized cell line. The transfected immortalized cell line containing the recombinant molecule is cultured to enable expression of the heterologous protein or polypeptide in the cell line. The methods employed in the design of the recombinant molecule, selection of the heterologous protein and regulatory sequences, and incorporation thereof into the cell line are within the skill of the art. [See, e.g., Maniatis et al., Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Press, Cold Spring Harbor New York (1989)].

Exemplary suitable vectors or plasmids for transfecting the immortalized tick cells include those with an operational promotor, including insect viruses, insect recombinant plasmids, Entomopox virus, and arboviruses. Currently, it is expected that insect promoters, such as polyhedron of baculovirus, spheroidin of entomopox virus, drosophila promoters or arbovirus (Semliki Forest Virus) would produce the best results in the immortalized tick cells. Similarly, vector components for mammalian cells and known vectors may be readily selected by one of skill in the art for use in transfecting the bovine cell line Bpbl-T1. See, for example, Maniatis et al, cited above.

The production of a desirable antigen from an immortalized tick cell line of this invention is exemplified below (Example 3). Analogous procedures may be employed to produce antigens in the immortalized bovine cell line of the invention. Media and cells from pathogen-infected immortalized cells are generally collected at 24-108 hours post-infection and are used as the source of antigen for immunizations. If desirable, antigen-containing media can be clarified from cell associated material by centrifugation, aliquoted and stored at -20°C until used. Alternatively, the pathogen antigenic material may remain associated with any cell material from the immortalized cell and be incorporated into a vaccine. Immortalized tick cell produced material in

association with the selected pathogen antigen may enhance the immunostimulatory effect of the pathogen antigen. All antigen preparations can be quantified for parasite-specific protein (PSP) with ELISA. Cell-associated antigen may be prepared by sonication on ice in serum-free media followed by the centrifugation step described above. Protein concentrations are determined by the method of Bradford, Anal. Biochem., 72:248 (1976). Sonicated parasite suspensions are adjusted to a final concentration of 10-100 μg/mL in serum-free media, aliquoted, and stored at -20°C until use.

The pathogens may express receptors on the cell surface of the immortalized cell of the invention or intracellularly. Alternatively, the pathogens are contained intracellularly and released when the immortalized cell is disrupted in vitro or is processed in vivo by the animal vaccinated with the pathogen-containing immortalized cell. Disruption may be accomplished using known means, e.g. by freeze-thawing or other biochemical or mechanical disruption. In still another alternative, antigenic portions of the pathogen are purified or left in combination, i.e. the antigens may comprise a mixture of unpurified cellular material, viral subunits or fragments, media, and, optionally an adjuvant, from the immortalized cell in which they are produced and used in a vaccine formulation.

The antigens produced as described above can be employed in the preparation of a vaccine. The vaccine comprises an immunogenic amount of one or more antigen produced by the invention in a form suitable for internal administration.

Such a vaccine, directed against the selected pathogen or parasite, comprises an immunogenic amount of at least one pathogenic antigen which is produced by growing an immortalized cell culture of this invention infected with the selected pathogen, including viruses and microorganisms. The pathogenic antigen, as defined above, includes the entire pathogen, desirable subunits, polypeptides, cell fractions or fragments thereof, or desirable non-proteinaceous material.

Alternatively, the vaccine contains a whole immortalized cell and the pathogen antigen. The pathogenic antigen may be expressed in the immortalized cell intracellularly, on the cell surface, or secreted during the natural processing of the cell or a vaccine formulation containing a pathogen-infected immortalized cell by the host. Alternatively, such a vaccine composition may contain some cellular component of the immortalized cell which is not the whole cell, e.g., an immunogenic protein or polypeptide fragment of the immortalized cell, a subunit non-proteinaceous material, or mixtures thereof.

In another embodiment, a vaccine is designed to protect against a tick. Such a vaccine composition contains an immunogenic amount of an immortalized tick cell

of the invention, a cellular fraction, an antigenic protein or fragment of the cell, or other suitable immunogenic fragments of an immortalized cell of the invention. In another embodiment, the anti-parasite vaccine of the invention may contain both of the immortalized tick cell lines of the invention, or combinations thereof, whether as whole cells, cellular fractions, or only proteinaceous or desirable non-proteinaceous materials from the cells. Further, these vaccine compositions may include other conventional anti-parasitic agents suitable for internal administration and/or be administered in connection with other, known tick vaccinal compositions. Example 2 illustrates cross-species reactivity with an anti-tick vaccine of the invention.

The AGEC-1 and DGEC-1 immortalized tick cell lines of the present invention and the Bpbl-T1 bovine cell line are used to prepare vaccines with or without incorporated and replicating pathogens (See Example 3) and a pharmaceutically acceptable carrier. For example, as a tick vaccine, the uninfected or non-pathogen associated tick cells are replicated to the desired volume and cell density using large scale cell culture procedures known to those in the art such as (e.g., roller bottles, microcarrier, suspension, hollow fiber, etc.). The cells are harvested by standard procedures and concentrated by ultrafiltration or centrifugation. The cells are inactivated by 1-3 cycles of freeze-thaw, or heat or suitable chemical inactivation. The inactivated cells are then adjuvanted under optimal conditions to provide a suspension of cells and adjuvant. The bovine cells may be analogously manipulated for the preparation of a vaccine composition.

In another formulation, the membrane proteins of the immortalized cells harvested can be fractionated by standard methods to form a vaccine containing only a part of the immortalized cell of the invention alone or in combination with other antigens. This vaccine would not need an inactivation step, but may optionally be adjuvanted and administered in the manner described above. Similarly, one of skill in the art can identify desirable immunogenic proteins, peptides, or polypeptides derived from an immortalized cell of the invention for inclusion in a vaccine composition of the invention. Such proteins, peptides, or polypeptides once identified, can be isolated and purified, produced recombinantly, or synthesized by known means. Vaccines of this invention may be employed in a method of immunizing humans or animals against a selected pathogen by injecting a vaccine of this invention into the animal.

As one example, a vaccine composition may contain as one of its active ingredients a selected Borrelia antigen produced in cell line AGEC-1 or DGEC-1, and either purified from the cell line or used in association with cellular material from the tick cell line. Such a vaccine is desirably administered to humans and animals to

stimulate immunity against Lyme disease or ticks. Where the vaccine comprises Borrelia antigens only, the vaccinee may develop immunity against the spirochete, the causative agent of Lyme disease. Where the vaccine also contains tick cell antigenic material, the vaccinee may develop an immune response which destroys the tick before it can transfer the spirochete to the vaccinee. In the same manner, a vaccine of this invention can be used to protect against Rocky Mountain spotted fever, Ehrlichiosis, arboviruses, anaplasmosis, theileriosis, babesiosis, cutaneous irritations, and allergic dermatitis, where the antigen produced in the cell line is derived from one of the pathogens causing that condition.

Alternatively, where a vaccine of the invention is formulated to be protective against Lyme disease, the whole immortalized tick cell, or a cellular fraction or subunit, a tick cell protein or peptide fragment, or combination thereof, from the immortalized cells may provide one vaccine component to stimulate immunity to the tick cell. A tick cell vaccine component may be separately administered with a vaccine containing a whole, inactivated pathogenic cell or subunit vaccine prepared from Borrelia. In other words, the Borrelia antigen does not have to be produced in the immortalized cells. It may be produced in another conventional way and co-administered with an immortalized tick cell component-containing composition. Alternatively, in the present invention, Borrelia are grown in the immortalized cells and then the entire immortalized cells and spirochete material are harvested, inactivated, and preferably adjuvanted as described above. Another embodiment involves separating the spirochete or spirochete-produced proteins or antigenic fragments from the immortalized cells after growth and preparing the spirochetes or proteinaceous fragments separately from the cells as a vaccine. In this case, the spirochete is inactivated, or a protein isolated and adjuvanted in a similar manner.

Thus, the present invention provides a vaccine useful in preventing infection with any tick-borne pathogen. Such a vaccine composition contains an immortalized tick cell of the invention, or an immunogenic or antigenic fragment thereof, and an antigen directed to the selected pathogen. This antigen may be a whole inactivated viral or cellular pathogen, or an antigenic protein, macromolecule or polypeptide thereof. For example, in a preferred embodiment, an anti-Lyme disease vaccine contains both the immortalized cells and any Borrelia burgdoferi antigen. An example of such an antigen is the spirochete, described in Example 9. However, this invention is not limited to an antigen produced in association with the immortalized tick cells of the invention, but the Borrelia antigen may be obtained from other sources. It is anticipated that such a vaccine would offer superior protection over a vaccine containing solely the spirochete or subunits thereof.

An immortalized cell of the invention can also be used as a recombinant vector for expression of a gene from a selected pathogen, e.g., Borrelia. Thus the need for growing and maintaining the spirochete would be eliminated. Such a recombinant vector expressing the gene for Borrelia is introduced into the immortalized tick cells. The tick cells expressing the gene are harvested as described above, inactivated, adjuvanted, and optimized for stability and efficacy.

For example, certain Borrelia express outer surface proteins (OSPs) which are capable of differentially distinguishing between Borrelia grown in standard laboratory media and Borrelia passaged through live ticks. Using a recombinant DNA approach, genes representing the OSPs from Borrelia vectored by live ticks could be isolated and expressed in an appropriate vector. Particularly useful vectors include the Semliki forest virus vector, the baculovirus vector and the entomopox virus vector. The immortalized tick cells of the invention, infected with these viruses to produce the desired antigen are processed as whole cell or subunit extracts as described above.

Typically, antigenic proteins produced in association with the immortalized cells of this invention are desirably employed in vaccine compositions. For example, an immunogenic amount of an antigenic protein or desirable non-proteinaceous material are mixed with a pharmaceutically acceptable carrier. An immunogenic amount of a selected pathogenic antigen is generally between about 0.01 μg to 10.0 mg antigen, more preferably 0.05 μg - 1 mg, and may be determined by one of skill in the art depending on the identify of the antigen, pathogen, and host animal.

An immunogenic amount of the immortalized tick cells is about 10^1 to 10^8, and preferably about 10^6 cells/dose. Alternatively, the total immortalized cell proteinaceous material, cellular fractions, or desirable non-proteinaceous macromolecules is in the range of 0.05 μg to 1 mg, as described above for the pathogenic antigens above. However, one of skill in the art can make appropriate adjustments depending upon the vaccine.

In addition to the active ingredients discussed in the preceding paragraphs, other optional ingredients including, for example, stabilizers, carriers, and adjuvants may be added to the vaccine compositions of the invention. Stabilizers are added optionally to provide longer shelf life or enhance the potency of the formulated vaccines of the invention. Typically, stabilizers, adjuvants, and inactivation agents are optimized to determine the best formulation for efficacy in the target animal.

Suitable stabilizers, as with the other optional ingredients, are well known to those of skill in the art. Examples of such stabilizers include casamino acids, sucrose, gelatin, phenol red, N-Z amine AS, monopotassium glutamate, potassium

monophosphate, potassium diphosphate, bovine albumin fraction V, lactose, lactalbumin hydrolysate, dried milk, and heat inactivated serum.

Suitable pharmaceutically acceptable carriers facilitate administration of the proteins and antigens but are physiologically inert and/or nonharmful. Carriers may be selected by one of skill in the art. Exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextrin, agar, pectin, peanut oil, olive oil, sesame oil, and water. Additionally, the carrier or diluent includes a time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax. In addition, slow release polymer formulations can be used.

The vaccine compositions of this invention may be incorporated into conventional sustained-release matrices, implant formulations, tablet formulations or injectable sustained release formulations and matrices. Components of such formulations are well known to the art.

One or more of the above described vaccine components may be admixed or adsorbed with a conventional adjuvant. The adjuvant is used as a non-specific irritant to attract leukocytes or enhance an immune response. Such adjuvants include, among others, mineral oil and water, aluminum hydroxide, Amphigen, Avridine, L121/squalene, D-lactide-polylactide/glycoside, pluronic polyols, muramyl dipeptide, killed Bordetella, saponins, saponin-derivatives such as Quil A, and Immune Stimulatory Complexes (ISCOMs).

Inactivation agents may also be included in the vaccine formulations of the invention. Suitable inactivation agents include formalin, glutaraldehyde, binaryethyleneimine (BEI), beta-propriolactone, and heat, and freeze/thaw techniques. Other suitable agents are well known in the art.

A desirable dosage of a vaccine of the invention involves the administration of 1 to 3 doses of desired vaccine composition, where the desired antigenic content of each fraction is as stated herein. The vaccine is preferably administered in two injections intramuscularly or subcutaneously, approximately 2 weeks to 3 months apart to immunize and boost the immune system of the target animal. However, the mode of administration of the vaccines of the invention may be any suitable route, including intradermally, intravenously, intraperitoneally, transdermally, topologically (e.g. by patch ointment) and by implant.

The specific dose level, mode and timing of administration for any particular animal depends upon a variety of factors including the age, general health and diet of the animal; the species of the animal; and the degree of protection being sought. Of course, the administration can be repeated at suitable intervals, such as annually, if necessary or desirable.

For example, in cattle, the volume of the dose may vary from 0.1 to 5 mL of a sterile preparation of an immunogenic amount of the active vaccine component or components to 0.1 to 1 mL in a small animal such as a dog or cat. The immune measurements to detect active immunization of the target animal can be determined by testing titers of antibody to cell protein using in vitro detection systems such as ELISA.

In another aspect, the immortalized cells of the invention may provide significant therapeutic products. The appropriate medium and other conditions under which to culture an immortalized cell line of this invention to enable it to produce a desired therapeutic agent can be readily determined by one of skill in the art by conventional techniques. Such a therapeutic agent may be secreted directly into the culture medium and isolated by conventional techniques by one of skill in the art. Alternatively, if expressed internally, the agent can be isolated from a culture lysate of such cells using known techniques.

Thus, the present invention also provides a method of immunizing animals against tick infestation, Lyme disease, and other tick-borne diseases by administering to an animal a vaccine compostion of the invention. For the method of immunizing animals against tick infestation, the vaccine composition of the invention contains an immunogenic amount immortalizing tick cell of the invention, DGEC-1, AGEC-1, progeny or derivatives thereof, or combinations of these cells. For the method of immunizing animals against Lyme disease, the vaccine composition of the invention may contain an immunogenic amount of one or more B. burgdorferi antigen produced in, on, or by an immortalized tick cell line of the invention, an immortalized tick cell line of the invention alone or in combination with one or more B. burgdorferi antigens, or a combination of these.For the method of immunizing animals against a selected tick-borne disease, the vaccine composition of the invention may contain an immunogenic amount of one or more antigen from the pathogen which causes the tick-borne disease produced in, on, or by an immortalized tick cell line of the invention, an immortalized tick cell line of the invention alone or in combination with one or more of the pathogen, or a combination of these.

The present invention further provides a method of immunizing animals against a selected pathogen, where said pathogen is capable of being grown in association with the immortalized bovine T cells of the invention. In particular, bovine leukemia virus, bovine immunodeficiency virus or other pathogens which infect bovine lymphocytes are anticipated to be suitable pathogens for growth in the Bpb1-T1 immortalized bovine T cells.

The immortalized cells of the invention also provide a source of diagnostic reagents. For example, the DGEC-1 or AGEC-1 immortalized tick cell lines of the invention, or preferably protein sequences therefrom, may be used as diagnostic reagents in an in vitro assay to detect the presence of a pathogen, particularly a tick-borne pathogen, in a sample of body fluids from an animal suspected of infection. In addition, pathogens produced in the immortalized cells of the invention, and their protein sequences may also provide diagnostic agents for the pathogen. Further, the specificity of the immortalized cell receptors can be used to determine typing of organisms isolated from patients suspected to have been exposed to such organisms. Once the receptor is identified, receptor specific typing can be performed.

Such a diagnostic assay preferably involves the association of the cell line of the invention with a detectable label and the incubation of this diagnostic reagent with the sample fluids. For example, the specificity of the receptors on the immortalized tick cell sof the invention, can be used to type organisms isolated from patients suspected of being bitten by a tick. Because the DGEC-1 cell specifically agglutinates strain 297 of Borrelia burgdorferi, agglutination could be used to type other B. burgdorferi strains. Binding of a pathogen to the cells can be detected by a variety of conventional assays, including agglutination, amplification, stimulation of a pathogen antigen, e.g., OSP on Borrelia, and competition binding utilizing a MAb to the pathogen, e.g., Borrelia, or a receptor on an immortalized cell line of the invention.

In one embodiment, B. burgdorferi specifically agglutinates after attachment to DGEC-1 cells in vitro, indicating a specific receptor on the B. burgdorferi and DGEC-1 cells. After several days of attachment, the B. burgdorferi are induced to replicate. Normally, B. burgdorferi will not replicate in DGEC-1 media. The binding, agglutination and detachment denotes an activation (synergy) step between B. burgdorferi and DGEC-1. Thus, the immortalized cells and/or B. burgdorferi may elaborate growth factors in the media.

Antibodies to a receptor(s) on the immortalized tick cell lines of the invention, DGEC-1 or AGEC-1, may be used in diagnostic assays. Conventional techniques for making suitable polyclonal, recombinant, or more desirably, monoclonal antibodies are well known to those of skill in the art [see, e.g., Kohler and Milstein; W. D. Huse et al., Science, 246:1275-1281 (1988); PCT Patent Publication No. PCT/WO86/01533, published March 13, 1986; British Patent Application No. GB2188638A, published October 7, 1987; Amit et al., Science, 233:747-753 (1986); Queen et al., Proc. Natl. Acad. Sci. USA, 86:10029-10033 (1989); PCT Patent Publication No. PCT/WO90/07861, published July 26, 1990; and Riechmann et al.,

Nature, 332:323-327 (1988)]. For diagnostic purposes, the antibodies may be associated with individual labels, which are preferably interactive to produce a detectable signal. Most preferably, the signal is visually detectable. For colorimetric detection, a variety of enzyme systems have been described in the art which will operate appropriately.

Antibodies specific for receptors on DGEC-1 or AGEC-1 may also be used therapeutically as targeting agents to deliver therapeutic compounds. Rather than being associated with a label, such a therapeutic agent employs the antibody linked to an agent or ligand capable of disabling the replicating mechanism of the pathogen. Alternatively, the antibody may block binding of the pathogen to the target cell. Such antibodies may also be useful in therapeutic compositions for treating tick infestations.

The following examples are merely illustrative of the different aspects of this invention and are not intended to limit the scope of the present invention.

Example 1 - Characterization of Transformed Cell Line

The immortalized cells DGEC-1 derived from the intestinal epithelial tract of Dermacentor andersoni show continuous growth and replication by a budding process, which can result in "chain-like" clusters of cells. Growth characteristics change with increased duration in culture. However, replication rates among gut cells transformed according to this method vary, as does the ease of transformation. At approximately nine months after initiation of cultures, the cell replication rate increased and slight morphological changes occurred. A distinct nucleus became visible for the first time. It is possible that cells required this time to accommodate to culture conditions and to delete transfected DNA, which is not needed for cellular growth and development.

Three types of cells have been observed in the heterogeneous population: small, round and clear; large, granular and brown; and large clear cells. Cells possess phagocytic activity throughout the culture period. Antimicrobials have been removed from established cultures without any detectable changes in cellular growth characteristics.

The DGEC-1 cell line was further characterized by (1) selective attachment by Borrelia burgdorferi (receptor mediator), (2) differential phagocytosis of red blood cells in mixed blood cell populations and (3) induction of immune response in guinea pigs that reduced tick infestation and feeding. The line has remained stable for over 15 months.

Further, this cell line, as well as immortalized intestinal epithelial cells from Amblyomma americanum (AGEC-1), have proven useful in maintaining in vitro cultures of A. marginale, B. burgdorferi, and Ehrlichia species. It is expected that these cell lines will also be useful in maintaining in vivo cultures of Arboviruses, Babesia, and other tick-borne pathogens.

The growth of certain of these pathogens is demonstrated in Example 3 below.

Example 2 - Immunization of Guinea Pigs with Immortalized Tick Gut cells

Successful vaccination of guinea pigs with tick gut extracts is always accompanied with a specific antibody titer than can be enhanced upon repeat vaccinations. These antibody titers are normally measured by ELISA tests. To determine whether the immortalized gut cells have retained the ability to synthesize important antigens for immunological control of ticks, the cells were used to vaccinate guinea pigs. Three parameters are used to determine whether the guinea pig has been successfully immunized against tick infestation: (a) death during or shortly after the blood meal is taken, (b) reduction or elimination of ovipositing (egg laying), (c) reduction or lack of hatching from egg mass that is laid. These parameters are additive in the clinical measurements against tick infestation since any can block the tick life cycle. This example describes the results of vaccination and challenge studies in the guinea pig model and demonstrates production of antibody against the immortalized tick cells that cross react with native tick gut tissue in an ELISA format.

The continuously growing tick gut cells of the invention were harvested from L-15B complete medium, centrifuged (at 150 x g; 10 minutes), and washed 3x in L-15B incomplete medium. The cells were then resuspended in L-15B incomplete medium and adjusted to 1.0×10^6 cells/mL. One million cells were administered by subcutaneous injection to each guinea pig on days 0 and 14. Each injection was in a volume of 1 mL, and given in a dose divided between two sites. A total of 12 guinea pigs: 6 for Amblyomma americanum cells and 6 for Dermacentor andersoni cells were used. Two weeks after the last injection of cells, the animals were bled by cardiac puncture, 4 mL of blood from each animal was collected, serum separated and stored at -20°C. Immunized animals developed strong antibody responses as determined by enzyme linked immunosorbent assay (ELISA) as described below.

The antigen was prepared as follows. Adult, female, unfed Dermacentor andersoni and Amblyomma americanum (200 each) were surface sterilized and their gut tissue was collected in sterile PBS (pH 7.2, 0.15 M). The gut preparation was

sonicated and the protein content determined by bicinchoninic (BCA) assay as described in P. K. Smith et al, Analytical Biochemistry, 150:76-85 (1985).

The coating for the wells was made up of 5 µg/mL of gut antigen suspended in carbonate-bicarbonate buffer, loaded 100 µl/well, and incubated at 4°C overnight. The wells were blocked with 1% bovine serum albumin.

Serum dilutions (both control and immune) were made from 1:10 to 1:20480. The second antibody used was rabbit anti-guinea pig (IgG, H&L) in HRPO, 1:1000 in 1% BSA (PBS, 0.05% Tween-20). The substrate was o-phenylene dramine [OPD; Eastman Kodak, Rochester, NY] and the optical density was read at 490 nm. The results of these assays are set out in Tables 1-3 below.

Tables 1 and 2 set out the results of an assay performed on guinea pigs immunized with Amblyomma americanum gut cells immortalized by the technique described in Example 5 above. The native gut antigen (5 µg/mL) was 500 ng/well in 100 µL as determined by enzyme-linked immunosorbent assay, ELISA, using known techniques. Tick gut protein concentrations were determined prior to testing as a standard. Each well was coated identically, each using the same antibody dilution and detector antibody. Labelled antibody was rabbit anti-guinea pig-HRPO (IgG, H&L), 1:1000. The pre-immunization titers (OD_{160}) of A. americanum antigen were 0.037 and of D. andersoni antigen were 0.064.

Table 1 represents the determination of antibody response in animals immunized with immortalized A. americanum cells (AGEC-1) and not challenged with ticks. The time periods indicate the time after inoculation at which the sera was tested. The antigen refers to the antigen used in the test.

Table 1

	FIVE WEEK POST-INJ. NATIVE GUT ANTIGEN		SIX WEEK POST-INJ. NATIVE GUT ANTIGEN	
Guinea Pig	Amblyomma	Dermacentor	Amblyomma	Dermacentor
1009	1.313	1.187	1.267	1.306
1010	1.144	1.036	1.230	1.292
1011	0.687	0.785	1.064	1.047

The following data represents the determination of antibody response by ELISA in animals immunized with immortalized A. americanum cells and which were tick challenged.

Table 2

	NATIVE GUT ANTIGEN	
Guinea Pig	Amblyomma	Dermacentor
1018	0.326	0.340
1019	0.545	0.667
1020	0.459	0.711
1021	0.381	0.203
1022	0.303	0.429
1023	0.461	0.613

Single cell suspensions were prepared from D. andersoni freshly isolated gut. The isolated cells were frozen and thawed. Guinea pigs were immunized with this preparation at 0.5 mg per mL per animal. A total of three booster injections were given with a 10 day interval between inoculations. As can be seen from the data in Table 3 below, animals immunized with the immortalized Dermacentor andersoni gut cells (DGEC-1) developed strong antibody responses. The antigen of Table 3 is that used for screening.

Table 3

	NATIVE GUT ANTIGEN	
Guinea Pig	Amblyomma	Dermacentor
1024	0.167	0.343
1025	0.208	0.334
1026	0.160	0.442
1027	0.617	0.947
1028	0.495	0.458
1029	0.485	0.468

The antibodies generated against freshly prepared tick gut tissue by immunization of guinea pigs were used in immunofluorescence assays to detect reactive epitopes on immortalized tick gut cells maintained in culture for 11 months. These guinea pig antibodies were obtained from animals that were subsequently challenged with ticks. After collection of sera for immunolocalization, immunized animals were challenged with larvae, nymphs or adults of the sensitizing species. This immunolocalization data correlates with the protective study results.

Table 4 below illustrates the effect on the life cycle patterns of Dermacentor andersoni ticks on control animals and may be compared with Table 5 which illustrates similar data on animals immunized with Dermacentor andersoni native gut antigen.

Table 4

	#infest/ #replete	Feeding days	Weight	Dead/live	Physical Appearance
Larvae	280/275	4		5/275	Hatched
Nymphs	60/60	6		0/60	Molted
Adults	8/8	8-10	946 mgs* 1034 mgs* 1023 mgs* 943 mgs* 1045 mgs* 988 mgs* 1038 mgs* 1011 mgs*	0/8	

Table 5

	#infest/ #replete	Feeding days	Weight	Dead/ live	Physical Appearance
Larvae	230/216	4		14/216	
Adults	8/4	18-19	392 mgs 435 mgs 643 mgs 572 mgs	4/4	

This data shows that animals immunized with freshly prepared D. andersoni gut cells were resistant to infestation with adult ticks. None of the four female ixodids allowed to infest immunized guinea pigs produced ova, indicating a clear anti-tick response.

Tables 6 and 7 below illustrate this effect on Amblyomma americanum and Dermacentor andersoni ticks in animals immunized with Amblyomma americanum gut cells immortalized by the technique described above.

Table 6 - A. americanum ticks

	#infest/ #replete	Feeding days	Weight	Dead/ live	Physical Appearance
Larvae 1018	220/253	4	228 mg/0.9 mg	n[1]	Molted
Nymphs 1019	60/45 (75.0%)	6	572 mg/12.71 mg	n[2]	Molted
Adults					
1020	8/8	12	23 mg		
		12	434 mg	Dead	Reddish
		13	690 mg		Reddish
		14	55 mg		Dark red
		19	35.6 mg	Dead	Dark red
		19	34.8 mg	Dead	Dark red
		19	15.6 mg	Dead	Dark red
	(100 % dead)				

n[1] (100% fed larvae survived)

n[2] {45 Molted to adults (75.0%), 15 dead (25.0%)}

The red color indicates that the tick guts apparently disintegrated. One tick burst in capsule. The adult ticks in this study either died or were incapable of laying eggs. None produced egg mass.

Table 7 - D. andersoni ticks

	#infest/ #replete	Feeding days	Weight	Dead/ live	Physical Appearance
Larvae 1021					
Nymphs 1022	60/52 (86.7%)	8	1421 mg/27.33 mg	23/29 n[1]	29 grey 23 black
Adults			(Tick)	(Egg Mass)	
1023	8/8	12	485 mg	244.9 mg*	Dark red
		12	792 mg	523.0 mg	Grey
		12	579 mg	358.6 mg*	Grey
		13	786 mg	465.8 mg	Grey
		13	539 mg	235.9 mg	Grey
		13	405 mg	198.9 mg	Grey
		14	498 mg	323.6 mg	Grey
		15	250 mg		Grey

n[1] {17 molted to adult (28.3%), 43 dead (81.8%)}

* For the adults, indicates that the ova produced by females did not hatch.

The lack of data for larvae indicates that they did not attach to the host. However, while there was no feeding, a severe cutaneous reaction was seen at the bite site. No egg mass was produced for those feeding 15 days. The data further indicates that the immunization induced cross-species protection which is indicated by longer times to replete, lower finished weight and reduced viable egg mass.

Tables 8 and 9 below illustrate this effect on Amblyomma americanum and Dermacentor andersoni ticks in animals immunized with immortalized Dermacentor andersoni gut cells obtained by the technique described above.

Table 8 - D. andersoni ticks

	#infest/ #replete	Feeding days	Weight	Dead/ live	Physical Appearance
Larvae:					
1024					
Nymphs:					
1025	60/38 (63.3%)	8	1058 mg/27.84 mg	n[1]	31 grey 7 black
Adults:					
1026	8/6	12	768 mg	dead	black
		12	972 mg	dead	dark red
		12	791 mg*		grey
		12	936 mg**		grey
		13	970 mg	dead	reddish
		19	15.6 mg		grey

n[1] {25 molted to adults (41.7%), 35 dead (58.4%)}
* Indicates the production of egg mass (324.6 mg), which did not hatch.
** Indicates the production of 353.5 mg egg mass.

Two ticks died early during feeding period. The lack of data for larvae indicates that there was no attachment. However, while no feeding occurred for larvae, a severe cutaneous reaction was seen at the bite site.

Table 9 - A. americanum ticks

	#infest/ #replete	Feeding days	Weight	Dead/ live	Physical Appearance
Larvae 1027	200/210	4	174 mg/0.83 mg		Molted
Nymphs 1028	60/38	6	366 mg/9.63 mg		all grey
	{37 molted to adults (61.7%), 23 dead (38.4%)}				
Adults 1029	8+8	14	296 mg		dark red
		14	370 mg*		grey
		14	417 mg**		grey
		15	276 mg	dead	black
		18	158 mg		dk grey
		18	315 mg		dk grey
		19	37.5 mg		grey
		19	23.4 mg	dead	black

* Indicates production of 131.5 mg egg mass.
** Indicates the production of 167.4 mg egg mass.

Animals immunized with immortalized cells were challenged with larvae, nymphs or adults of the homologous species. Preliminary observations indicated that resistance to adult and nymphal challenge was present. Adult ticks required longer to feed and engorgement was altered. Many male, female and nymphal ticks died after engorgement. In addition, production of ova was reduced. Immortalized digestive tract cells of A. americanum appear to stimulate a more solid resistance to infestation than cells of D. andersoni origin.

This data indicates that the immunization induced some cross-species protection.

Example 3 - Growing Pathogens in Immortalized Tick Cells

A. Ehrlichia canis and Granulocytic Canine Ehrlichia

Both Ehrlichia canis and Granulocytic canine Ehrlichia (GCE) have been successfully grown in the immortalized gut cells of Dermacentor andersoni (DGEC-1) and Amblyomma americanum (AGEC-1). The Ehrlichia was obtained from peripheral blood collected from dogs which were experimentally infected with these organisms. E. canis is associated with lymphocytes and monocytes. GCE is associated with granulocytes. The peripheral blood was collected in vacutainers containing heparin.

Peripheral blood lymphocytes (PBL) were isolated by Ficoll gradient (Histopaque) and washed 3x in L-15B incomplete medium. The cell numbers were then adjusted to 1.0×10^6 cells/mL and added to a flask containing gut cells (6 mL) (a few erythrocytes also were present in the peripheral blood lymphocyte preparations.

Granulocytes were isolated from blood by the method described by Sigma Chemicals using Histopaque 1077 and 1119 in a double gradient method. Isolated granulocytes were washed 2x in L-15B incomplete medium inoculated to gut cells (1.0×10^6 cells/mL).

Microscopic examination of cultures 2-3 days post infection showed aggregation of PBL/granulocytes around the gut cells. This was very evident in Amblyomma americanum gut cells, since they are big, oval and larger than PBLs/granulocytes. About 2-3 weeks post-inoculation, the contaminated RBC degenerated, PBL/granulocytes showed rough surfaces and numerous granular material was observed in cultures. In addition, Amblyomma cells showed phagocytosis of hemoglobin (appeared red in color).

Eight to ten weeks after infecting cells, the minute bodies increased in number and many of these bodies studded on to the surface of the gut cells, particularly on Amblyomma americanum gut cells. Diff-Quick stained culture smears and one micron thin sections (toluidine blue stained) showed numerous bodies (lawn of uniform bodies), which were also studded on the surface of the gut cells.

Serum samples collected from dogs suffering from Ehrlichiosis were used as a source of antibody and used to identify the organisms in cultures by Florescent Antibody Test. Acetone fixed smears of culture were reacted with antibodies to Erhlichia, and these immunoglobulins were localized by fluoroscein isothyocyante (FITC) conjugated anti-canine antibodies.

Cultures with material from infected dogs displayed reactivity. Immortalized gut cells had the appearance of being studded with minute fluorescent bodies.

B. Borrelia burgdorferi

Borrelia burgdorferi strain 297 [SmithKline Beecham Animal Health] was grown in conventional BSK-II medium according to known techniques. Strains B31 [SmithKline Beecham] and SH-2-82 [National Institutes of Health] of B. burgdorferi have also been established. Two vector competent strains include 27985, isolated from I. dammini in Stamford, Connecticut, and 21305, which was isolated from Peromysus leucopus [both of which are avaiiable from John Anderson, Connecticut Agricultural Resource Station]. However, other, known, strains of B. burgdorferi may be used.

An 8-10 day old culture of B. burgdorferi strain 297 (200 µL) was added to each of two T-25 flasks. Dermacentor andersoni immortalized gut cells (DGEC-1) and Amblyomma americanum immortalized gut cells (AGEC-1) were cultured in antibiotic-free L-15B complete medium 24-48 hours prior to inoculating with the spirochetes. The spirochete infected tick cell cultures were incubated at 30° C in a dry incubator as described below.

1. Dermacentor andersoni

The spirochetes started attaching to the cells by 24 hours post infection. As the incubation continued, the clumping of cells by spirochetes increased. By 4-5 days post infection, clear entanglement of cells by spirochetes was evident. It was observed that the cell types in the culture, small, round, clear gut cells were attacked by the spirochetes. The large, round, brown color cells may be resistant to spirochete attachment. By 10-12 days post infection, the spirochete sensitive cells were disintegrated and only spirochete resistant cells were present in the culture. By 15 days post infection, the so-called spirochete resistant cells started multiplying and increasing in number. By 20 days post infection, the spirochete resistant cells were seen uniformly throughout the flask.

Spirochetes also showed multiplication. The number of spirochetes increased. The spirochete resistant cells may provide some growth factors for the multiplication of spirochetes.

After 12 days of culturing spirochetes in the cells, spirochetes were passaged in a new flask containing gut cells. In the first passage, the clumping of the cells was not seen until 4-5 days post infection. In this flask also, the spirochete attachment was seen only with small, round, clear cells. The large, round, brown cells were not affected by spirochetes.

2. Amblyomma americanum

These gut cells showed similar spirochete attachment. The only difference observed in this system was that clumping and attachment of spirochetes to cells was seen only after 4-5 days. The large, oval, brown cells were not attacked by the spirochetes.

C. Anaplasma marginale

This organism was obtained from peripheral blood collected from cattle suffering from anaplasmosis or A. marginale-infected cattle in vacutainers containing heparin and which showed 27% parasitemia as determined by blood smear examination.

The buffy coat of A. marginale was removed by centrifugation and erythrocytes were washed 2x in L-15B incomplete medium. The red blood cell

number was adjusted to 1.0×10^7 cells/mL in L-15B complete medium with all growth factors without antibiotics. Flasks containing approximately 5 mL of immortalized Dermacentor andersoni and Amblyomma americanum gut cells were inoculated with 1.0×10^6 cell/mL infected erythrocytes (2 flasks for each cell type). After inoculation, the cells were incubated at about 30°C as described below.

Twenty-four hours post-inoculation of red blood cells (RBC) into the culture, gut cells showed attachment of RBC onto the surface gut cells. This was more evident in Amblyomma than the Dermacentor cells (in both cell types these attachments are seen). Forty-eight hours post-inoculation there was phagocytosis of RBC by gut cells. The cell membrane of the gut cells became thick and numerous; RBC were studded onto the surface of the gut cells. In addition, these gut cells appeared dark brown.

Five days post-inoculation, the gut cells appeared highly granular, contained numerous minute uniform inclusion bodies, and many cells ruptured and released these inclusion bodies into the surrounding medium. Culture samples were collected for transmission electron microscopy for analysis of infected gut cells. Changes were more evident in the Amblyomma cells.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

WHAT IS CLAIMED IS:

1. An immortalized tick cell line AGEC-1, ATCC Accession No. CRL 11083, its progeny and derivatives thereof.

2. The immortalized cell line according to claim 1 infected with a selected pathogen.

3. The immortalized cell line according to claim 1 transfected with DNA from a selected pathogen encoding a selected gene product under the control of regulatory sequences capable of expressing said gene product.

4. An immortalized tick cell line DGEC-1, ATCC Accession No. CRL 11084, its progeny and derivatives thereof.

5. The immortalized cell line according to claim 4 infected with a selected pathogen.

6. The immortalized cell line according to claim 4 transfected with DNA from a selected pathogen encoding a selected gene product under the control of regulatory sequences capable of expressing said gene product.

7. An immortalized bovine T cell line Bpbl-T1, ATCC Accession No. CRL-11120, its progeny and derivatives thereof.

8. The immortalized cell line according to claim 7 infected with a selected pathogen.

9. The immortalized cell line according to claim 8 transfected with DNA from a selected pathogen encoding a selected gene product under the control of regulatory sequences capable of expressing said gene product.

10. An isolated pathogenic antigen produced by culturing an immortalized tick cell selected from the group consisting of AGEC-1, DGEC-1, progeny and derivatives thereof infected with a selected pathogen.

11. An isolated pathogenic antigen produced by culturing an immortalized bovine T cell line selected from the group consisting of Bpbl-T1, progeny and derivatives thereof infected with a selected pathogen.

12. An antigen produced by culturing an immortalized tick cell selected from the group consisting of AGEC-1, progeny and derivatives thereof transfected with DNA from a selected pathogen encoding a selected gene product under the control of regulatory sequences capable of expressing said gene product.

13. An antigen produced by culturing an immortalized tick cell selected from the group consisting of DGEC-1, progeny and derivatives thereof transfected with DNA from a selected pathogen encoding a selected gene product under the control of regulatory sequences capable of expressing said gene product.

14. An antigen produced by culturing an immortalized bovine T cell selected from the group consisting of Bpbl-T1, progeny and derivatives thereof, transfected with DNA from a selected pathogen encoding a selected gene product under the control of regulatory sequences capable of expressing the gene product.

15. A vaccine capable of protecting against infection with a selected pathogen comprising an immunogenic amount of at least one pathogenic antigen produced by culturing an immortalized tick cell selected from the group consisting of AGEC-1, DGEC-1, progeny and derivatives thereof infected with said selected pathogen.

16. A vaccine capable of protecting against infection with a selected pathogen comprising an immunogenic amount of a pathogenic antigen produced by culturing an immortalized bovine T cell selected from the group of cell lines consisting of Bpbl-T1, progeny and derivatives thereof infected with the selected pathogen.

17. The vaccine according to claim 16 wherein the pathogenic antigen is produced by transfecting said immortalized bovine T cell with DNA from the selected pathogen encoding the selected antigen under control of regulatory sequences capable of expressing the pathogenic antigen.

18. A vaccine capable of protecting against infection with a selected tick-borne pathogen and against infestation by a tick, wherein said vaccine composition comprises an immunogenic amount of an antigen obtained from an immortalized tick cell selected from the group consisting of AGEC-1, DGEC-1, progeny and derivatives thereof and an antigen from the selected tick-borne pathogen.

19. The vaccine according to claim 18 wherein the selected tick-borne pathogen is selected from the group consisting of *Borrelia burgdorferi*, *Amblyomma americanum*, *Anaplasma marginale*, *Babesia bovis*, *Theileria parva*, *Cowdria ruminantium*, Ehrlichia species, and arboviruses.

20. The vaccine according to claim 19 wherein said pathogenic antigen is produced by transfecting said immortalized tick cell with DNA from said selected pathogen encoding a selected antigen under the control of regulatory sequences capable of expressing said antigen.

21. The vaccine according to claim 18 wherein said tick cell antigen is selected from the group consisting of a whole immortalized tick cell, a cellular fraction or subunit thereof, a protein and a macromolecule from said tick cell.

22. The vaccine according to claim 18 wherein the selected pathogen is produced by infecting the immortalized tick cell line with said pathogen.

23. A vaccine capable of protecting against tick infestation comprising an immunogenic amount of an antigen obtained from an immortalized tick cell selected from the group consisting of AGEC-1, DGEC-1, progeny and derivatives thereof.

24. The vaccine according to claim 21 wherein said tick cell antigen is selected from the group consisting of a whole immortalized tick cell, a cellular fraction or subunit thereof, a protein and a macromolecule from said tick cell.

25. A method of immunizing an animal against a disease caused by a selected tick-borne pathogen comprising internally administering to said animal a vaccine, wherein said vaccine composition comprises an immortalized tick cell antigen, said immortalized tick cell selected from the group consisting of AGEC-1, DGEC-1, progeny and derivatives thereof and an antigen from said selected pathogen.

26. The method according to claim 25 wherein said pathogen is *Borrelia burgdorferi* and said disease is Lyme disease.

27. A method of immunizing an animal against tick infestation comprising the step of internally administering to said animal a vaccine comprising an immunogenic amount of an antigen obtained from an immortalized tick cell selected from the group consisting of AGEC-1, DGEC-1, progeny and derivatives thereof.

28. The vaccine according to claim 27 wherein said antigen is selected from the group consisting of (a) a whole immortalized tick cell, (b) a cellular fraction of said immortalized tick cell, (c) an immunogenic protein or macromolecule of said immortalized tick cell, and (d) a mixture of any one of (a), (b) and (c).

29. A method of immunizing an animal against a disease caused by a selected pathogen comprising internally administering to the animal a vaccine composition comprising an immortalized bovine T cell selected from the group consisting of Bpbl-T1, progeny and derivatives thereof transfected with DNA from the selected pathogen encoding the selected antigen under control of regulatory sequences capable of expressing the antigen.

30. A method of immunizing an animal against a disease caused by a selected pathogen comprising internally administering to the animal a vaccine composition comprising an immortalized bovine T cell selected from the group consisting of Bpbl-T1, progeny and derivatives thereof infected with the selected pathogen.

31. A method of detecting the presence of a tick-borne pathogen in the body fluids of an animal comprising the step of incubating a body fluid sample taken from the animal in the presence of an antigen selected from the group consisting of (a) a whole immortalized tick cell selected from the group consisting of AGEC-1, DGEC-1, progeny and derivatives thereof, (b) a cellular fraction of said immortalized tick

cell, (c) an immunogenic protein or fragment of said immortalized tick cell, and (d) a mixture of any one of (a), (b) and (c).

32. An antibody specific for a receptor on an immortalized tick cell selected from the group consisting of AGEC-1, DGEC-1, progeny and derivatives thereof.

33. An antibody specific for a receptor on an immortalized bovine T cell selected from the group consisting of Bpbl-T1, progeny and derivatives thereof.

34. A therapeutic composition useful for treating tick infestations comprising an antigen selected from the group consisting of (a) a whole immortalized tick cell selected from the group consisting of AGEC-1, DGEC-1, progeny and derivatives thereof, (b) a cellular fraction of said immortalized tick cell, (c) an immunogenic protein or fragment of said immortalized tick cell, and (d) a mixture of any one of (a), (b) and (c).

35. A diagnostic reagent comprising an antigen selected from the group consisting of (a) a whole immortalized tick cell selected from the group consisting of AGEC-1, DGEC-1, progeny and derivatives thereof, (b) a cellular fraction of said immortalized tick cell, (c) an immunogenic protein or fragment of said immortalized tick cell, and (d) a mixture of any one of (a), (b) and (c).

36. A diagnostic reagent comprising an antigen selected from the group consisting of (a) a whole immortalized bovine cell selected from the group consisting of Bpbl-T1, progeny and derivatives thereof, (b) a cellular fraction of said immortalized bovine cell, (c) an immunogenic protein or fragment of said immortalized bovine cell, and (d) a mixture of any one of (a), (b) and (c).

INTERNATIONAL SEARCH REPORT

Inte..ational application No.
PCT/US93/08606

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/00; C12N 5/06, 5/10, 15/00; C07K 15/04, 15/28; G01N 33/53
US CL :424/88, 93B; 435/7.1, 69.3, 70.3, 240.2; 530/387.1, 350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 93B; 435/7.1, 69.3, 70.3, 240.2; 530/387.1, 350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, MEDLINE, BIOSIS, EMBASE, DERWENT,
search terms: tick, cell, bovine, amblyoma, dermacentor, gut

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Infection and Immunity, Volume 58, No.4, issued April 1990, M.E. Brandt et al, "Immunogenic Integral Membrane Proteins of *Borrelia burgdorferi* are Lipoproteins", pages 983-991, see entire document.	10-14
X,P	US, A, 5,186,933 (Estes) 16 February 1993, see entire document.	15-17
X Y	Journal of Immunological Methods, Volume 128, Number 2, issued 17 April 1990, E.J.Glass et al, "Generation and Characterization of Bovine Antigen-Specific T Cell Lines", pages 267-275, see entire document.	7,33 30,36

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
11 November 1993

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08606

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Veterinary Immunology and Immunopathology, Volume 14, issued 1987, R.J. Scibienski, "Establishment and Initial Characterization of Continuous In Vitro Cultures of Bovine T Lymphocytes", pages 209-222, see entire document.	7,33 30,36
X Y	Veterinary Immunology and Immunopathology, Volume 23, Number 3-4, issued December 1989, M.J. Romano et al, "Phenotypic Characterization of Bovine Lymphoblastoid Cell Lines", pages 293-307, see entire document.	7,33 30,36
X Y	Immunology, Volume 69, No.2, issued February 1990, H. Takamatsu et al, "Characterization of Long-Term Cultured Bovine CD4-Positive and CD8-Positive T-Cell Lines and Clones", pages 293-297, see entire document.	7,33 30,36
Y	US, A, 4,447,537 (C.E. Yunker et al) 08 May 1984, see entire document.	1,2,4,5,8,18-22,25-29,31,34,35
X Y	Medical Science Research, Volume 15, issued 1987, S.K. Wikel et al, "Immunization Induced Resistance to *Amblyomma americanum* Infestation: Tick Gut Derived Antigens", pages 543-544, see entire document.	23,24,27,28,32,34 18-22,25,26,31,35
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